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INHERITANCE OF SOME SEED-COAT COLORS AND PATTERNS IN LIMA BEANS¹

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A GREAT DIVERSITY OF SEED-COAT COLORS AND PATTERNS occurs in the lima bean species, *Phaseolus lunatus* L.; among the most common colors are white, red, purple, and black, while the most common patterns are self-colored and mottled. The Mendelian basis of the inheritance of these colors is imperfectly known, the only report in the literature being that of Rhind (1933), who described two color genes and one pattern gene. According to Rhind, the gene pair *Rr* governs rose vs. white color, and the dominant allele of the gene pair *Pp* is an intensifier which darkens the rose color of *R* to purple, but is inactive in the presence of *rr*. Rhind reported that the gene pair *Ss* is incompletely dominant, *Ss* producing light (restricted) speckling, *Ss* heavy (diffuse) speckling, and *ss* self-color.

The investigations of seed-coat color reported here were conducted at Davis, California, during the period 1947-1952. These studies have contributed additional evidence regarding the inheritance of seed-coat color and have also established the relationships of the seed-coat color genes to some genes governing hypocotyl and flower color (Allard, 1952).

MATERIALS AND METHODS

The four parents with white seed coats used in these studies are the widely grown commercial varieties Henderson, Green-Seeded Henderson, Wilbur, and Westan. The colored parents are all unnamed strains maintained in the University of California lima-bean collection. The seed-coat colors and patterns are given in table 1. Color designations follow the Munsell system.³

In table 1 and thereafter, a single color designation indicates that the seed coat is self-colored. Mottled seed coats are indicated by a dual color designation. As an example, red/buff indicates red mottling on a buff background color. The two types of mottling observed, restricted and diffuse (fig. 1), are distinguished by underscoring. Thus red/buff indicates restricted red mottling on a buff background, and red/buff indicates diffuse mottling involving the same colors.

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³ Munsell Book of Color. Munsell Color Co., Baltimore, Md. 1942.

TABLE 1
SEED-COAT COLORS OF PARENTAL VARIETIES OR STRAINS, ACCORDING
TO THE MUNSELL SYSTEM

Variety or strain	Munsell color		Color description
	Mottle	Background	
Wilbur.....	9.0N	White
Westan.....	9.0N	White
Henderson.....	9.0N	White
Green-Seeded Henderson.....	9.0N	White
L37.....	2.5R 3/8	Red
L44.....	5.0R 2/2	Red
L75.....	2.5R 2/6	Red
L49.....	7.5RP 2/2	Dark red
L124.....	2.5R	Dark red
L76.....	5.0P 3/4	Purple
L111.....	5.0P 3/4	Purple
L50.....	2.5R 3/10	2.5R 5/8	Red/red
L126.....	2.5R 4/6	7.5YR 8/4	Red/buff
L54.....	10.0R 2/2	2.5R 3/10	Dark red/red
L48.....	1.0N	7.5YR 7/4	Black/buff
L122.....	1.0N	7.5YR 7/4	Black/buff
I166-7-1.....	5.0P 3/4	2.5R 2/6	Purple/red
I166-7-2.....	5.0P 3/4	7.5YR 8/4	Purple/buff 1
I166-7-3.....	10.0R 3/2	2.5R 5/8	Dark red/red
I166-7-8.....	1.0N	10.0R 2/2	Black/red



Fig. 1. Left: Restricted mottling produced by the genotype *SS*. Right: Diffuse mottling produced by the genotype *Ss*.

When F_3 or F_4 progenies were grown to identify the genotype of F_2 or F_3 plants, minimum progeny sizes necessary to distinguish between the expected ratios at odds 99:1 were calculated in order that the appropriate number of seeds might be planted in each progeny. In certain progenies inadequate numbers were obtained to permit the positive identification of genotypes, and an additional planting the following season was necessary.

EXPERIMENTAL RESULTS

Hybrids of White × Red

In three hybrids (table 2) between parents with white and red seed coats, the F_1 seed-coat color in each case was red. The ratios observed in F_2 indicate that a single gene pair governs this color difference (table 2). Additional

TABLE 2

OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
3 RED:1 WHITE IN THE F_2 OF HYBRIDS BETWEEN PARENTS
WITH RED AND WHITE SEED COATS

Hybrid	Red, number	White, number	χ^2	Probability exceeds
Wilbur × L37.....	342	99	1.53	0.20
Green-Seeded Henderson × L75.....	171	75	3.95	0.02
Green-Seeded Henderson × L44.....	168	69	2.13	0.10

TABLE 3

OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
9 DARK RED:3 RED:4 WHITE IN THE F_2 OF HYBRIDS BETWEEN
PARENTS WITH DARK-RED AND WHITE SEED COATS

Hybrid	Dark red, number	Red, number	White, number	χ^2	Probability exceeds
Green-Seeded Henderson × L49.....	66	17	32	1.36	0.50
Green-Seeded Henderson × L124.....	201	74	88	0.65	0.70

evidence was obtained from a small F_3 of the hybrid Green-Seeded Henderson × L75. Among 30 families derived from red-seeded F_2 parents, 12 were homozygous for red seed-coat color, and 18 segregated approximately 3 red:1 white—an acceptable fit to the expected 1:2 ratio. Ten F_2 plants with white seed coats produced only white-seeded F_3 progeny.

Hybrids of Dark Red × White

The seed-coat of the F_1 in the two hybrids studied (table 3) was indistinguishable from that of the colored parents. In the F_2 generation, three seed-coat colors occurred, the parental colors and red. Although there was considerable variation in the hue, chroma, and value within the two colored classes, little difficulty was encountered in distinguishing between the dark-red and the red groups. The proportion of dark red:red:white was 9:3:4, indicating that the parents differ in two gene pairs. One of these gene pairs apparently governs red *vs.* white seed-coat color, and the other intensifies the red color to dark red but is not expressed except in the presence of at least one dominant allele of the red-white pair.

A total of 89 F_3 families from the hybrid Green-Seeded Henderson × L124 were grown to test this hypothesis. Among the 20 F_3 progenies derived from

F_2 parents with red seed coats, 7 were homozygous for red seed-coat color, and 13 families segregated approximately 3 red:1 white. This is a good fit to the 1:2 ratio expected. The 1:2:2:4 ratio expected among the F_3 progeny of F_2 plants with dark-red seed coats was also realized (table 4). Ten F_2 plants with white seed coats produced only white-seeded progeny. Thus F_3 data confirm the Mendelian scheme deduced from the F_2 segregation.

The hypocotyl color of both of the dark-red-seeded parents, L49 and L124, is red, while that of the Green-Seeded Henderson parent is green. It is known that red hypocotyl color in lima beans is produced by the coöperation of the dominant alleles of the gene pairs *Cc* and *Rr* (Allard, 1952), resulting in ratios of 9 red:7 green in F_2 . In the F_2 of the present hybrids, the ratios of plants with red hypocotyls to plants with green hypocotyls were 146:139 for the hybrid Green-Seeded Henderson \times L49 and 150:141 for the hybrid of

TABLE 4

OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
1:2:2:4 AMONG THE PROGENIES OF F_2 INDIVIDUALS WITH
DARK-RED SEED COATS

Hybrid	Homo. dark red, number	3 Dark red : 1 red, number	3 Dark red : 1 white, number	9 : 3 : 4, number	χ^2	Probability exceeds
E.T. \times L124.....	2	12	11	34	5.908	0.10

Green-Seeded Henderson \times L124. These are acceptable fits to a 9:7 ratio. Because hypocotyl color can be classified accurately only in seedling stages, and seed-coat color only at maturity, it was necessary to mark seedling plants to establish correlations between hypocotyl and seed-coat colors. When this was done it was found that plants with red hypocotyls invariably had dark-red seed coats and that green hypocotyl color was associated with red or white seed-coat color. It is apparent that the basic color factor and the red-pigmentation gene pairs involved in the production of red hypocotyl color are identical with the red and dark-red seed-coat color genes, respectively, of the present hybrids. Using the symbols applied to the hypocotyl-color genes (Allard, 1952), the colored parents, L49 and L124, are genotypically *CCRR*, and the Green-Seeded Henderson parent is genotypically *ccrr*. It is suggested that *Cc* be adopted as the symbol for the basic color factor, despite the priority of Rhind's symbol, because it is more descriptive of the action of this gene.

Hybrids of White \times Purple

The seed-coat color of the four F_1 hybrids studied (table 5) was indistinguishable from that of the purple parents. In the F_2 generation three seed-coat colors—purple, red, and white—occurred in a ratio of 9:3:4 (table 5). Although there was considerable variability within the two colored classes, the discontinuity between the two groups was clearly demarcated. Among the F_3 progenies of 20 F_2 plants with red seed coats, six were homozygous for red, and 14 segregated approximately 3 red:1 white. A 1:2:2:4 ratio was observed among F_3 progenies of purple-seeded F_2 plants (table 6).

By marking F_2 plants in seedling stages it was established that plants with purple hypocotyls invariably had purple flowers and purple seed coats, whereas plants with green hypocotyls had white flowers and either red or white seed coats. Purple pigmentation of the hypocotyl, flowers, and seed coat thus results from manifold effects of the same purple-pigmentation gene.

TABLE 5

OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
9 PURPLE:3 RED:4 WHITE IN THE F_2 OF HYBRIDS BETWEEN
PARENTS WITH PURPLE AND WHITE SEED COATS

Hybrid	Purple, number	Red, number	White, number	χ^2	Probability exceeds
Wilbur \times L76.....	223	60	94	2.14	0.30
Green-Seeded Henderson \times L76.....	187	74	99	2.71	0.20
Westan \times L76.....	132	52	79	5.03	0.05
Green-Seeded Henderson \times L111.....	73	21	43	3.30	0.10

TABLE 6

OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
1:2:2:4 AMONG THE PROGENIES OF F_2 INDIVIDUALS WITH
PURPLE SEED COATS

Hybrid	Homo. purple, number	3 Purple : 1 red, number	3 Purple : 1 white, number	9 : 3 : 4, number	χ^2	Probability exceeds
E.T. \times L76.....	4	11	16	20	2.735	0.30

TABLE 7

OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
3:6:3:4 IN THE F_2 OF HYBRIDS BETWEEN PARENTS WITH
RED/RED AND WITH WHITE SEED COATS

Hybrid	Red/red, number	Red/red, number	Red., number	White, number	χ^2	Probability exceeds
Green-Seeded Henderson \times L50.....	38	68	42	46	1.38	0.70

This gene is identical with the purple-hypocotyl gene described by Allard (1952) and is apparently the same as the intensifier gene of Rhind (1933). The genetic formula for the purple parents in these hybrids is thus $CCPP$, and the formula for the white-seeded parents is $ccpp$.

Hybrid of White \times Red/Red

A single hybrid of this type was studied (table 7). It was expected that the red/red parent would carry the basic color factor CC and the mottling (speckle) gene, SS , of Rhind (1933), and Green-Seeded Henderson is known from the hybrids previously reported to be genotypically $ccss$. These predictions were confirmed when the seed-coat color and pattern of the F_1 hybrid was red/red and when a good fit to a 3:6:3:4 ratio was obtained in the F_2 (table 7).

Hybrid of White × Red/Buff

The F_1 hybrid of Green-Seeded Henderson × L126 had seed coats red/buff in color and pattern. It was possible to identify six classes in the F_2 , as shown in table 8. The 9:18:3:6:12:16 ratio observed would be expected if the white parent were genotypically $ccsssrssr$ and the colored parent $CCSSSsSrSr$, where the $Srsr$ alleles govern the background color, $Sr-$ producing buff background and $srsr$ red background color in the presence of $S-$ but being inoperative in the presence of ss .

TABLE 8

OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
9:18:3:6:12:16 IN THE F_2 OF HYBRIDS BETWEEN PARENTS WITH
RED/BUFF AND WITH WHITE SEED COATS (GREEN-SEEDED
HENDERSON × L126)

Phenotype	Observed number	Expected number
Red/buff.....	31	30.8
Red/buff.....	58	61.6
Red/red.....	7	10.3
Red/red.....	15	20.5
Red.....	49	41.1
White.....	59	54.8
Total.....	219	219.1

$$\chi^2 = 4.55.$$

P exceeds 0.30.

A total of 45 colored F_3 families were grown to check this hypothesis. It was possible to study the segregation of the postulated $Srsr$ gene pair in 39 families with mottled seed coats. Six families derived from F_2 plants with red background color produced only parental types with respect to background color. Of the 33 F_3 families derived from F_2 plants with buff background color, 10 were homozygous for this type, and 23 segregated approximately 3 buff background:1 red background. These results indicate monogenic control of background color.

Six of the 45 families were derived from self-colored F_2 plants. They proved to be homozygous for self color and were judged to be genotypically ss . Similarly, eight families derived from parents with restricted mottling bred true for this characteristic, and were genotypically SS . The remaining 31 families were derived from parents with seed coats characterized by diffuse mottling; all 31 produced restricted mottled plants, diffuse mottled plants, and self-colored plants in a ratio of approximately 1:2:1. In none of the self-colored individuals was there any indication of modification of the seed-coat color by action of the $Srsr$ gene pair.

Among the 45 F_3 families, 18 were homozygous colored, and 27 segregated approximately 3 colored:1 white. This represents a good fit to the expected segregation at the Cc locus. Both F_2 and F_3 segregations thus support the genetic formulas for the parents predicted from the parental seed-coat colors.

The symbol $Srsr$ was chosen to indicate that this gene pair governs the background color associated with the Ss locus, modifying the buff background color to red.

Hybrid of White \times Dark Red/Red

It was deduced on the basis of the genes reported above that the F_1 of the hybrid between Green-Seeded Henderson and L54 should be genotypically $CcRrSssrsr$. All of the classes expected on the basis of this Mendelian proposition occurred in the frequencies expected in the F_2 generation (table 9). The segregation for hypocotyl color was determined in seedling stage, 136 red

TABLE 9

OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
9:18:9:3:6:3:16 IN F_2 OF THE HYBRID GREEN-SEEDED
HENDERSON \times L54 (WHITE \times DARK RED/RED)

Phenotype	Observed number	Expected number
Dark red/red.....	33	33.3
Dark red/red.....	69	66.7
Dark red.....	30	33.3
Red/red.....	7	11.1
Red/red.....	26	22.2
Red.....	10	11.1
White.....	62	59.3
	237	237.0

$\chi^2 = 2.82$.
P exceeds 0.80.

and 115 green individuals being observed, an acceptable fit to a 9:7 ratio. Complete correlation was found between red hypocotyl and dark-red seed-coat colors, and between green hypocotyl and red or white seed-coat colors.

Hybrid of White \times Black/Buff

One hybrid of this type, Westan \times L48, was studied. F_1 hybrid plants had red-purple hypocotyls and black/buff seed coats. The genetic control of hypocotyl color in this hybrid is known to be as follows (Allard, 1952): $C_R_P_-$ — red purple; C_R_pp — red; C_rrP_- — purple; cc_- or C_rrpp — green. By marking seedling F_2 plants of various hypocotyl colors, it was established that red-purple hypocotyl color was associated with black or black/buff seed coats, red hypocotyl color with dark-red or dark-red/buff seed-coat color, purple hypocotyl color with purple or purple/buff seed-coat color, and green hypocotyl color with red, red/buff, or white seed-coat color. This suggested that the genotypes of the parents are $CCRRPPSS$ (L48) and $ccrrppss$ (Westan).

The segregation for seed-coat color in F_2 supports the above Mendelian scheme (table 10). It should be noted that classification for the mottle gene, Ss , was on the basis of mottled vs. nonmottled only. This restricted classification was necessary because only single seeds were harvested from the ma-

jority of the F_2 plants, and the variability in the extent of mottling between seeds on some plants was sufficiently great so that a single seed did not allow accurate separation of SS from Ss individuals.

In order to establish certainly that four genes govern the differences between the parents, selected F_3 and F_4 progenies were grown. Since 55 different F_2 genotypes should be distinguishable from F_3 progeny tests, the number of progenies required to determine the statistical relationships between progenies was impractically large. Hence only a limited number of progenies representing the various seed-coat colors and patterns were selected for

TABLE 10
OBSERVED SEGREGATION AND GOODNESS OF FIT TO A RATIO OF
81:27:27:9:27:9:9:3:64 IN THE F_2 OF THE HYBRID
WESTAN \times L48

Phenotype	Observed number	Expected number
Black/buff.....	302	298.7
Black.....	78	99.6
Dark red/buff.....	109	99.6
Dark red.....	36	33.2
Purple/buff.....	101	99.6
Purple.....	27	33.2
Red/buff.....	37	33.2
Red.....	15	11.1
White.....	239	236.0
Total.....	944	944.2

$$\chi^2 = 8.899.$$

$$\bar{P} = 0.30-0.50.$$

further tests. From the segregation in F_3 it was possible to select families expected to give the critical 1- and 2-gene segregations in F_4 . The results, summarized in table 11, indicate no unexpected interactions between the Cc , Rr , and Pp gene pairs.

A number of other F_4 progenies were grown to test for possible interactions of the Ss gene pair with each of the pigmentation genes. Three families judged from their mottling pattern to be SS or ss and six families judged to be Ss were grown within each of the red, dark-red, purple, and black seed-coat color classes. Self-colored parents and parents with restricted mottling bred true in every case. Parents with diffuse mottling in every case produced progeny with restricted mottling, diffuse mottling, and self-colored seed coats in ratios of approximately 1:2:1. The results showed that the effect of the primary pigmentation genes was largely confined to determining the color of the mottled portions of the seed coat. The single exception involved the $C_R_P_S$ genotype (black/buff or black/buff). The background color accompanying black mottling was almost invariably slightly but distinctly different from the background color of plants with dark red, purple, or red mottling. According to the Munsell color system, this modification was usually from approximately 7.5YR 8/4 for nonblack mottled plants to approximately 7.5YR 7/4 for black mottled plants.

Additional Hybrids of Red \times Buff Background Color

The discovery that cooperative action of the *CRP* pigmentation alleles affects not only the color of the mottled portions of the seed coat but also

TABLE 11

OBSERVED SEGREGATION AND GOODNESS OF FIT TO VARIOUS MONOGENIC AND DIGENIC RATIOS WITHIN F₂ FAMILIES DERIVED FROM F₃ FAMILIES OF KNOWN GENOTYPE IN THE HYBRID WESTAN \times L48

F ₃ Parent	F ₃ Genotype	F ₄ Segregation	F ₄ Genotype	Observed number	Expected number	χ^2	P exceeds
Dark red	<i>CCRrpss</i>	Homo. dark red.....	<i>CCRrpss</i>	2	4	1.000	
		3 dark red : 1 red.....	<i>CCRrpss</i>	10	8	0.500	0.20
				12	12	1.500	
Purple	<i>CCrrPpss</i>	Homo. purple.....	<i>CCrrPPss</i>	4	4	0.000	
		3 purple : 1 red.....	<i>CCrrPpss</i>	8	8	0.000	.99
				12	12	0.000	
Black	<i>CcRRPPss</i>	Homo. black.....	<i>CCRRPPss</i>	3	4	0.250	
		3 black : 1 white.....	<i>CcRRPPss</i>	9	8	0.125	.50
				12	12	0.375	
Black/buff	<i>CCRRPpSS</i>	Homo. black.....	<i>CCRRPpSS</i>	3	4	0.250	
		3 black : 1 dark red.....	<i>CCRRPpSS</i>	9	8	0.125	.50
				12	12	0.375	
Black/buff	<i>CCRrPPSS</i>	Homo. black.....	<i>CCRrPPSS</i>	5	4	0.250	
		3 black : 1 purple.....	<i>CCRrPPSS</i>	7	8	0.125	.50
				12	12	0.375	
Black	<i>CCRrPpss</i>	Homo. black.....	<i>CCRrPPss</i>	1	2	0.500	
		3 black : 1 purple.....	<i>CCRrPPss</i>	6	4	1.000	.50
		3 black : 1 dark red.....	<i>CCRRPpss</i>	3	4	0.500	
		3 dark red : 1 red.....	<i>CCRrPpss</i>	8	8	0.000	
				18	18	2.000	
Black/buff	<i>CCRrPpSS</i>	Homo. black/buff.....	<i>CCRRPpSS</i>	1	2	0.500	
		3 black/buff : 1 purple/buff.....	<i>CCRrPPSS</i>	4	4	0.000	0.30
		3 black/buff : 1 dark red/buff.....	<i>CCRRPpSS</i>	2	4	1.000	
		9 black/buff : 3 dark red : buff : 3 purple/buff : 1 red buff.....	<i>CCRrPpSS</i>	11	8	1.125	
				18	18	2.625	

buff background color raised the question of the effect of these alleles on the red background color produced by *srsr*. Information bearing on this question was obtained from four hybrid combinations in which *Sr-* and *srsr* occurred in several genetic backgrounds. The F₂ segregations observed in these

hybrids, reported in table 12, provide further support for the monogenetic control of buff *vs.* red background color. Careful inspection of the seed-coat colors of both F_1 and F_2 individuals with buff background color confirmed that the change reported above in the background color accompanies black

TABLE 12
OBSERVED SEGREGATION AND GOODNESS OF FIT TO RATIOS OF 3:1
IN THE F_2 OF HYBRIDS BETWEEN PARENTS WITH BUFF
VS. RED BACKGROUND COLORS

Hybrid	F_2 Phenotypes	Observed number	Calculated number	χ^2	P exceeds
I166-7-1 × I166-7-2	Purple/buff.....	129	123.75	0.223	0.30
	Purple/red.....	36	41.25	0.668	
				0.891	
I166-7-8 × L122	Black/buff.....	170	168	0.024	0.70
	Black/red.....	54	56	0.071	
				0.095	
I166-7-1 × L122	Black or purple/buff.....	165	158.25	0.288	0.20
	Black or purple/red.....	46	52.75	0.864	
				1.152	
I166-7-3 × L122	Black or dark-red/buff.....	132	135.75	0.103	0.50
	Black or dark-red/red.....	49	45.25	0.310	
				0.413	

mottling. It also revealed no consistent differences in the red background color of individuals genotypically $C_rrppS_$, $C_R_ppS_$, or $C_rrP_S_$; however, the red background color of $C_R_P_S_$ individuals was definitely more purplish in hue. Although some variations in color existed among all genotypes, presumably as a result of the segregation of minor modifying genes, the usual background colors of the black mottled and nonblack mottled genotypes were, on the Munsell system, 10.0RP 4/6 and 2.5R 4/6, respectively.

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